PCT/EP2003/012830

Figure 1, HCV J4L6 genome wild-type cDNA sequence, reference accession number AF054247,

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				ttctggagga		
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				gttgctgggt		
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				ggcatgagac		
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1321	cacctacaac	agccctagtg	gtgtcgcagt	tgctccggat	cccacaagct	gtcgtggaca
1381	tggtggcggg	ggcccactgg	ggagtcctgg	cgggccttgc	ctactattcc	atggtaggga
1441	actgggctaa	ggttctgatt	gtggcgctac	tctttgccgg	cgttgacggg	gagacccaca
1501	cgacggggag	ggtggccggc	cacaccacct	ccgggttcac	gtcccttttc	tcatctgggg
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1741	aggggtgggg	ccccatcacc	tatactaagc	ctaacagctc	ggatcagagg	ccttattgct
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2041	ccccgtgtaa	catcgggggg	gtcggtaacc	gcaccttgat	ctgccccacg	gactgcttcc
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2161	gcctagtaga	ctacccatac	aggctttggc	actacccctg	cactctcaat	ttttccatct
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2401	ctggtttgat	ccatctccat	cagaacatcg	tggacgtgca	atacctgtac	ggtgtagggt
				agtacatcct		
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2881	aatactttat	caccagagcc	gaggcgcaca	tgcaagtgtg	ggtcccccc	ctcaacgttc
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3001	acatcaccaa	actcctgctc	gccatactcg	gcccgctcat	ggtgctccag	gctggcataa
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				tcttcatgaa		
				gggactgggc		
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				gcaagttcct		
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				tgccccaaga		
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				gagtctggcg		
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				acggaacgtt		
				actattccag		
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6781	tccaggtcgg	gctcaaccaa	tacttggtcg	ggtcgcagct	cccatgcgag	cccgaaccgg

6841	acgtaacag	t gcttacttco	atgctcacco	g atccctccca	cattacagca	gagacggcta
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6961	ctgcgcctt	tttgaaggc	g acatgcacta	a cccaccatga	ctccccggac	gctgacctca
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7561	ccgtgagtga	ggaggctagt	gaggatgtcg	tctgctgctc	aatgtcctat	acgtggacag
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7681	actetttget	gcgtcaccac	aacatggtct	acgccacaac	atcccgcagc	gcaagcctcc
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7921	tccggaacct	atccagcagg	gccgttaacc	acatccgctc	cgtgtgggag	gacttgctgg
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8041	aaccagagaa	gggaggccgc	aagccagctc	gccttatcgt	attcccagac	ctgggagttc
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8221	ggaaatcaaa	gaaatgccct	atgggcttct	catatgacac	ccgctgtttt	gactcaacgg
8281	tcactgagag	tgacattcgt	gttgaggagt	caatttacca	atgttgtgac	ttggcccccg
8341	aggccagaca	ggccataagg	tcgctcacag	agcggcttta	catcgggggt	cccctgacta
8401	actcaaaagg	gcagaactgc	ggttatcgcc	ggtgccgcgc	aagtggcgtg	ctgacgacta
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8641	cccccccgg	ggatccgccc	caaccagaat	acgacctgga	gctgataaca	tcatgttcct
8701	ccaatgtgtc	agtcgcgcac	gatgcatctg	gcaaaagggt	atactacctc	acccgtgacc
8761	ccaccaccc	ccttgcacgg	gctgcgtggg	agacagctag	acacactcca	atcaactctt
8821	ggctaggcaa	tatcatcatg	tatgcgccca	ccctatgggc	aaggatgatt	ctgatgactc
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9001	gtcttagcgc	atttacactc	cacagttact	ctccaggtga	gatcaatagg	gtggcttcat
9061	gcctcaggaa	acttggggta	ccacccttgc	gaacctggag	acategggee	agaagtgtcc
9121	gcgctaagct	actgtcccag	aaaaaaaaa	ccgccacttg	tggcagatac (ctctttaact

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9181	gggcagtaag	gaccaagctt	aaactcactc	caatcccggc	cgcgtcccag	ctggacttgt
9241	ctggctggtt	cgtcgctggt	tacagcgggg	gagacatata	tcacagcctg	tctcgtgccc
9301	gaccccgctg	gtttccgttg	tgcctactcc	tactttctgt	aggggtaggc	atttacctgc
9361	tccccaaccg	atgaacgggg	agctaaccac	tccaggcctt	aagccatttc	ctgtttttt
9421	tttttttt	tttttttt	tctttttt	tttctttcct	ttccttcttt	ttttcctttc
9481	tttttccctt	ctttaatggt	ggctccatct	tagccctagt	cacggctagc	tgtgaaaggt
9541	ccgtgagccg	catgactgca	gagagtgctg	atactggcct	ctctgcagat	catgt

Figure 2, codon optimised HCV Core polynucleotide

Figure 3, Codon optimised HCV NS3 polynucleotide

ATGGCCCCCATCACCGCCTACAGCCAGCAGACCCGGGGACTGCTCGGCTGCATCATCACCTC AAAGCTTCCTGGCCACCTGTATCAACGGAGTCTGCTGGACGGTGTACCATGGCGCCGGCAGC AAGACCCTCGCCGGGCCTAAGGGCCCCATCACCCAGATGTACACCAACGTGGACCAGGACCT GGTGGGCTGGCAGGCCCCCCGGGGCGAGGAGTATGACCCCATGCACCTGCGGGAGCTCTG ${\tt ACCTGTATCTGGTGACCAGACATGCCGATGTCATCCCGGTGAGGCGTCGCGGGGACAGTAGA}$ ${\tt GGGAGCCTGCTGAGCCCCCGTCAGCTACCTGAAGGGGTCCGTGGGCGCCCCTGCT}$ ${\tt AGGCCGTGGACTTTATCCCCGTGGAGAGCATGGAGACCACCATGCGCTCCCCCGTGTTCACC}$ GACAACAGCAGCCCCCCCCCGCCGTGCCTCAGACCTTCCAGGTCGCCCACCTCCATGCTCCGAC $\tt GGGCTCCGGGAAGTCCACGAAGGTGCCCGCCGCGTACGCGGCCCAGGGATACAAGGTGCTGG$ ATCGACCCCAACATCAGAACTGGCGTCCGGACCATCACAACCGGCGCTCCCATCACTTACTC TACCTACGGCAAGTTCCTGGCTGATGGGGGGGTGTAGTGGGGGGGCGCGTACGATATTATCATCT GCCAGGAGTGCCACTCTACCGACAGCACCACAATCCTGGGCATCGGCACCGTCCTCGACCAG GCTGAGACAGCGGGCGCCCGGCTGGTGGTGCTGGCCACGGCCACTCCCCCGGGCTCCGTCAC GGTGCCCCACCCCAATATCGAGGAGGTGGCCCTGAGCAACAACGGCGAGATCCCATTCTACG GCAAGGCTATCCCGATCGAGGCGATTAAGGGAGGCAGACATCTGATCTTCTGCCACAGCAAG AAGAAGTGCGACGAGCTCGCCCCAAGCTGAGCGGCCTCGGACTCAACGCCGTGGCTTACTA CAGACCGTGGACTTCAGCCTGGACCCCACCTTCACCATCGAGACCACCACAGTGCCCCAGGA TCGTGACCCCGGGCGAGCGCCCAGCGGCATGTTCGATAGTTCCGTGCTGTGCGAGTGCTAC GACGCCGGATGCGCGTGGTACGAGCTGACCCCGGCGGAGACCTCTGTCCGCCTGAGGGCTTA $\tt CTTGAATACCCCGGGCCTGCCCGTGTGCCAGGATCATCTCGAGTTCTGGGAATCCGTCTTCA$ CCGGCCTGACACATCGACGCCCATTTCTTGTCCCAAACCAAGCAGGCTGGCGACAATTTC GGATCAGATGTGGAAGTGCCTGATCCGCCTGAAGCCCACCCTGCATGGGCCCACCCCCTGC

ATGGCGTGCATGTCCGCTGACCTGGAGGTGGTCACCTGA

Figure 4, codon optimised HCV NS4B polynucleotide

Figure 5, codon optimised HCV NS5B polynucleotide

ATGTCCATGTCCTACACCTGGACCGGCGCCCTGATCACCCCCTGCGCCGCGAGGAGCAA GCTCCCGATTAACCCCCTGTCCAACTCTCTGCTCCGCCATCACAACATGGTGTATGCCACCA CCTCCCGCTCTGCGAGCCTCCGCCAGAAGAAGGTGACGTTCGACAGACTGCAGGTGCTGGAC GACCATTACAGGGACGTGCTGAAGGAAATGAAGGCCAAGGCTAGCACCGTGAAGGCCAAGCT GCTCAGCATTGAGGAGGCTTGCAAGCTGACCCCCCCCCACAGTGCTAAATCCAAGTTCGGCT ACGGCGCCAAGGACGTGAGGAACCTGTCCTCGCGCGCGTGTGAACCATATCCGCAGCGTGTGG GAGGACCTGCTCGAGGACACCGAGACCCCCATCGACAACCATCATGGCCAAGTCCGAGGT TGGGCGTGAGAGTCTGCGAGAAGATGGCCCTCTACGACGTGGTGTCCACCCTGCCGCAGGCC GTGATGGGGAGTTCCTACGGCTTCCAGTACAGCCCGAAGCAGAGGGTGGAGTTCCTGGTGAA CACGTGGAAGTCTAAGAAATGCCCCATGGGGTTCAGTTACGGAACAAGGTGCTTCGGGAGTA CTGTGACCGAATCCGATATCCGCGTGGAGGAGGAGCATCTACCAGTGTTGTGACCTCGCCCCC GAGGCGAGACAGGCCATCCGCTCCCTGACCGAGAGGCTGTATATCGGCGGCCCACTGACCAA CAGCAAGGGCCAGAACTGCGGCTATCGCCGTTGTCGGGCCTCCGGGGTGCTCACCACCTCTT GACTGCACCATGCTCGTGAACGGCGACGATCTGGTGGTGATCTGTGAGTCCGCGGGCACGCA GGAGGACGCGGCGCCTGCGGGCGTTCACAGAGGCCATGACACGCTACAGTGCCCCCCCG GCGACCCCCCCAGCCCGAATACGATCTGGAGCTCATCACTAGTTGCAGCTCGAACGTGTCT GTGGCCCATGACGCTTCTGGCAAACGGGTGTATTATCTGACGCGCGATCCCACCACCCCCCT CGCCAGAGCCGCGTGGGAGACAGCTCGGCACACCCCTGTGAACTCTTGGCTGGGCAACATCA TCATGTACGCCCCTACCCTGTGGGCTCGCATGATCCTGATGACCCACTTCTTCAGTATCCTC CTCGCTCAGGAGCAGCTGGAGAAGGCGCTCGACTGCCAGATCTACGGCGCCCTGCTATAGTAT ATAGTTACTCTCCTGGAGAAATTAACCGGGTGGCGAGCTGTCTGCGGAAGCTCGGCGTCCCC CCTCTGCGCGTTTGGCGGCATCGCGCCAGGAGTGTGAGGCCAAGCTGCTGAGCCAGGGCGG AAGGGCCGCCACCTGCGGCCGGTATCTCTTCAACTGGGCCGTGCGCACCAAGCTCAAGCTCA GGCGACATCTACCACTCCCTCAGCAGGGCGCCCCCCGCTGGTTCCCCCTGTGCCTGCT CCTGAGCGTCGGAGTCGGCATCTACCTGCTGCCCAACCGCTGA

Figure 6, Translation of HCV J4L6 genome (wild-type sequence)

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						DPRRRSRNLG
						GCSFSIFLLA
						QEGNSSRCWV
241	ALTPTLAARN	ASVPTTTIRR	HVDLLVGTAA	FCSAMYVGDL	CGSIFLVSOT	FTFSPRRHET
301	VQDCNCSIYE	GHVSGHRMAW	DMMMNWSPTT	' ALVVSQLLRI	POAVVDMVAG	AHWGVLAGLA
						IQLVNTNGSW
421	HINRTALNC	DSLQTGFFAA	LFYAHKFNSS	GCPERMASCR	PIDWFAOGWG	PITYTKPNSS
						ENETDVMLLN
		GCTWMNSTGF				
		YPYRLWHYPC				
		EWQILPCAFT				
		VCACLWMMLL				
781	RLAPGAAYAF	YGVWPLLLLL	LALPPRAYAL	DREMAASCGG	AVLVGLVFLT	LSPYYKVFLT
		TRAEAHMQVW				
		YFVRAQGLIR				
		VEPVVFSAME				
1021	QGWRLLAPIT	AYSQQTRGVL	GCIITSLTGR	DKNQVEGEVQ	VVSTATQSFL	ATCINGVCWT
1081	VYHGAGSKTL	AGPKGPITQM	YTNVDLDLVG	WQAPPGARSM	TPCSCGSSDL	YLVTRHADVI
		SLLSPRPVSY				
		TDNSTPPAVP				
		AHGIDPNIRT				
		TVLDQAETAG				
		LIFCHSKKKC				
1441	MTGFTGDFDS	VIDCNTCVTQ	TVDFSLDPTF	TIETTTVPQD	AVSRSQRRGR	TGRGRSGIYR
		MFDSSVLCEC				
		HFLSQTKQAG				
1621	PTPLLYRLGA	VQNEVILTHP	ITKYIMACMS	ADLEVVTSTW	VLVGGVLAAL	AAYCLTTGSV
1681	VIVGRIILSG	KPAVVPDREV	LYQEFDEMEE	CASQLPYIEQ	GMQLAEQFKQ	KALGLLQTAT
1741	KQAEAAAPVV	ESKWRALETF	WAKHMWNFIS	GIQYLAGLST	LPGNPAIASL	MAFTASITSP
1801	LTTQNTLLFN	ILGGWVAAQL	APPSAASAFV	GAGIAGAAVG	SIGLGKVLVD	ILAGYGAGVA
1861	GALVAFKVMS	GEVPSTEDLV	NLLPAILSPG	ALVVGVVCAA	ILRRHVGPGE	GAVQWMNRLI
1921	AFASRGNHVS	PTHYVPESDA	AARVTQILSS	LTITQLLKRL	HQWINEDCST	PCSGSWLRDV
7044 ·	MOUNDERS	FKTWLQSKLL	PRLPGVPFLS	CQRGYKGVWR	GDGIMQTTCP	CGAQIAGHVK
2101	NGSMRIVGPR	TCSNTWHGTF	PINAYTTGPC	TPSPAPNYSR	ALWRVAAEEY	VEVTRVGDFH
2101	I VIGMITDNV	KCPCQVPAPE	FFTEVDGVRL	HRYAPACKPL	LREDVTFQVG	LNQYLVGSQL

2161	PCEPEPDVTV	LTSMLTDPSH	ITAETAKERI	ARGSPDST.AC	GGAGOT,GADG	T. K እ ጥር ጥጣ፣ III I	
2221	SPDADLIEAN	LLWRQEMGGN	ITRVESENKV	VILDSFEPLH	AEGDEREISV	AAEILRKSRK	
2281	FPSALPIWAR	PDYNPPLLES	WKDPDYVPPV	VHGCPLPPTK	APPIPPPRRK	RTVVLTESNV	
2341	SSALAELATK	TFGSSGSSAV	DSGTATALPD	LASDDGDKGS	DVESYSSMPP	LEGEPGDPDL	
2401	SDGSWSTVSE	EASEDVVCCS	MSYTWTGALI	TPCAAEESKL	PINPLSNSLL	RHHNMVYATT	
2461	SRSASLRQKK	VTFDRLQVLD	DHYRDVLKEM	KAKASTVKAK	LLSIEEACKL	TPPHSAKSKF	
2521	GYGAKDVRNL	SSRAVNHIRS	VWEDLLEDTE	TPIDTTIMAK	SEVFCVQPEK	GGRKPARLIV	
2581	FPDLGVRVCE	KMALYDVVST	LPQAVMGSSY	GFQYSPKQRV	EFLVNTWKSK	KCPMGFSYDT	
2641	RCFDSTVTES	DIRVEESIYQ	CCDLAPEARQ	AIRSLTERLY	IGGPLTNSKG	QNCGYRRCRA	
2701	SGVLTTSCGN	TLTCYLKATA	ACRAAKLQDC	TMLVNGDDLV	VICESAGTQE	DAAALRAFTE	
2761	AMTRYSAPPG	DPPQPEYDLE	LITSCSSNVS	VAHDASGKRV	YYLTRDPTTP	LARAAWETAR	
2821	HTPINSWLGN	IIMYAPTLWA	RMILMTHFFS	ILLAQEQLEK	ALDCQIYGAC	YSIEPLDLPQ	
2881	IIERLHGLSA	FTLHSYSPGE	INRVASCLRK	LGVPPLRTWR	HRARSVRAKL	LSQGGRAATC	
2941	GRYLFNWAVR	TKLKLTPIPA	ASQLDLSGWF	VAGYSGGDIY	HSLSRARPRW	FPLCLLLLSV	
3001	GVGIYLLPNR						

Figure 7, p7313-ie

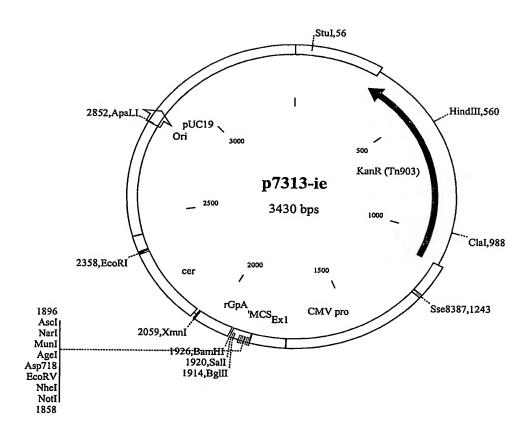
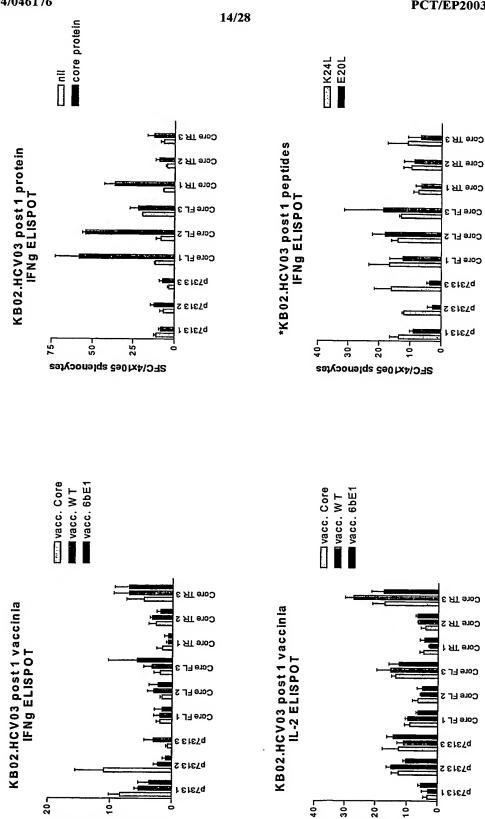


Figure 8, Immune responses to Core

SFC/4x10e5 splenocytes



SFC/4x10e5 splenocytes

1 TW ESN

E & 167q

D7313 S

r ereyq

150-

8

2FC/4x10e5 splenocytes

50-

LTW 62N

E E I E Y G

P7313 2

1 E 1 E 7 q

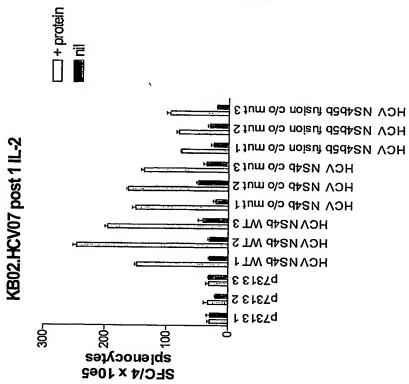
150

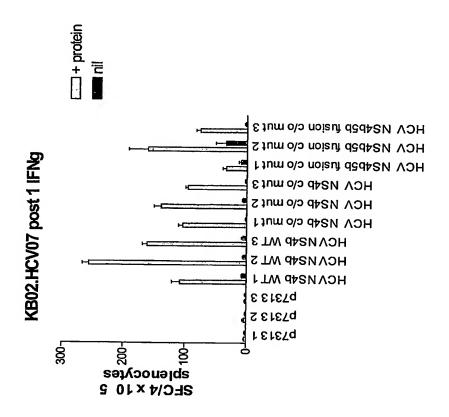
100

SFC/4x10e5 splenocytes

Figure 9, NS3 immunogenicity

Figure 10, Immune responses to NS4B





SFC/4x10e5 splenocytes

SFC/4x10e5 splenocytes

FIG. 12

Anti-HCV NS5B

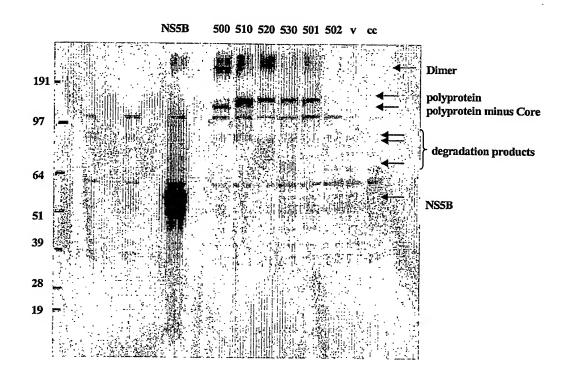
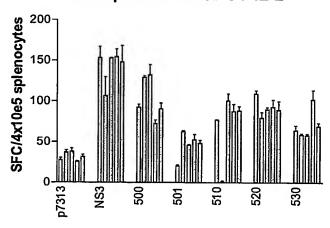


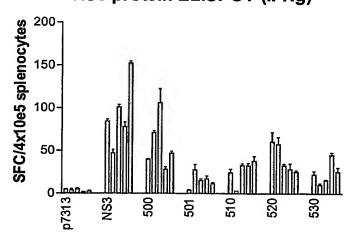
FIG. 13, A

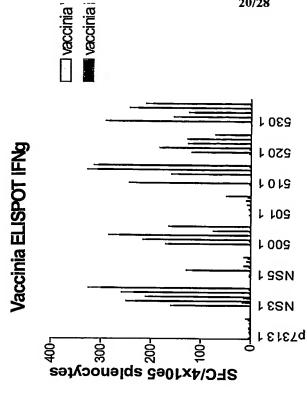
NS3 protein ELISPOT IL-2



В.

NS3 protein ELISPOT (IFNg)





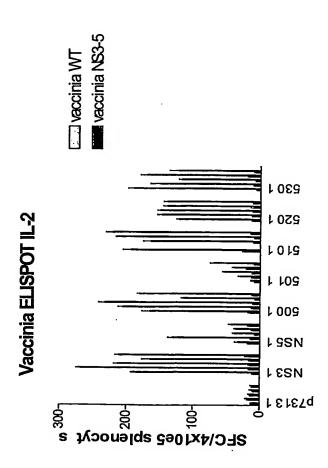


FIG 14.

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FIG. 15,

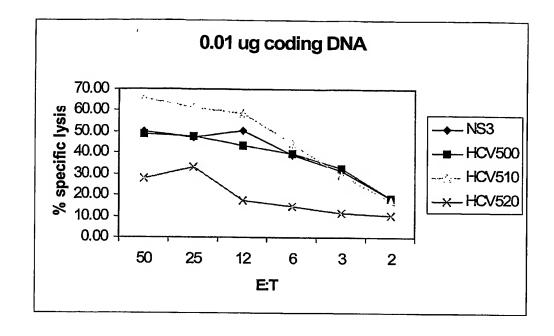




FIG. 16,

FIG. 17, C mparison of NS3 T cell response induced by dual promoter constructs.



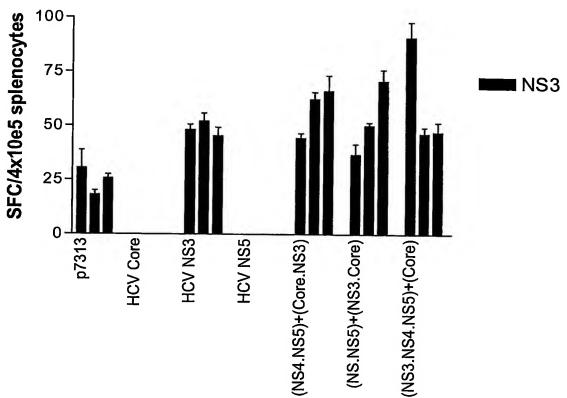
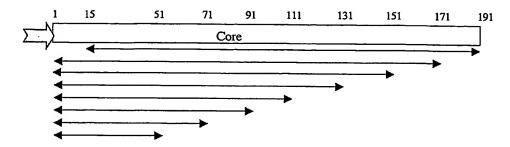


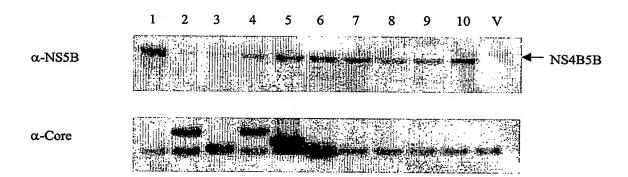
FIG. 18,



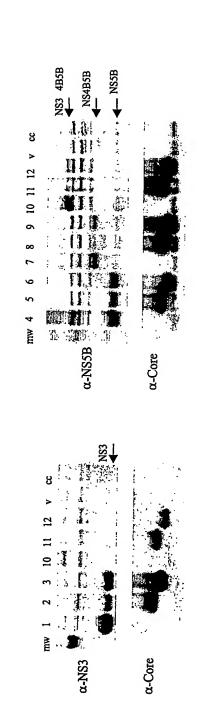
MW C191 CΔ15 C171 C151 C131 C111 C91 C71 C51



FIG. 19.



Effect of Core and Core, upon expression of NS3, NS5B, NS4B5B, and NS34B5B after co-transfection in 293T cells



11. p7/NS34B5B + p7/Core 12. p7/NS34B5B + p7/Core151 9. p7/NS4B5B + p7/Core151 8. p7/NS4B5B + p7/Core 10. p7/NS34B5B + v 7. p7/NS4B5B + v

> 3. p7/NS3 + p7/Core151 2. p7/NS3 + p7/Core

1. p7/NS3 + v Samples:

4. p7/NS5B + v

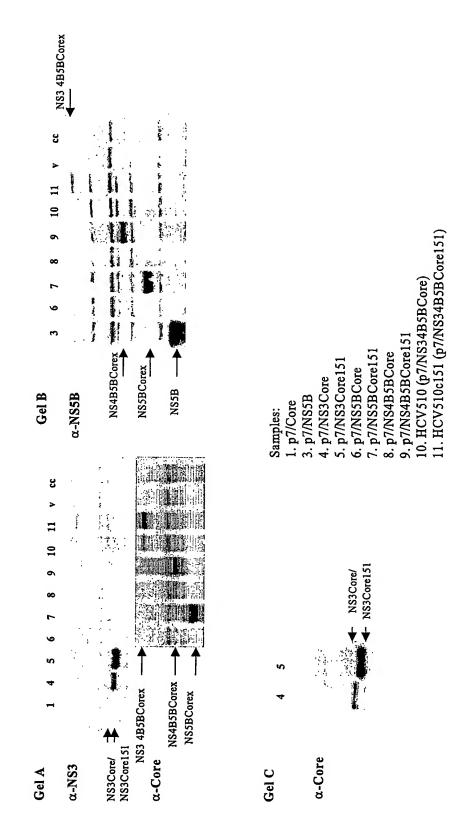
5. p7/NS5B + p7/Core 6. p7/NS5B + p7/Core151

FIG. 20,

PCT/EP2003/012830

FIG. 21,

Effect on expression of fusion proteins, after substitution of Cogg for Core₁₉₁, in transient transfection in 293T cells



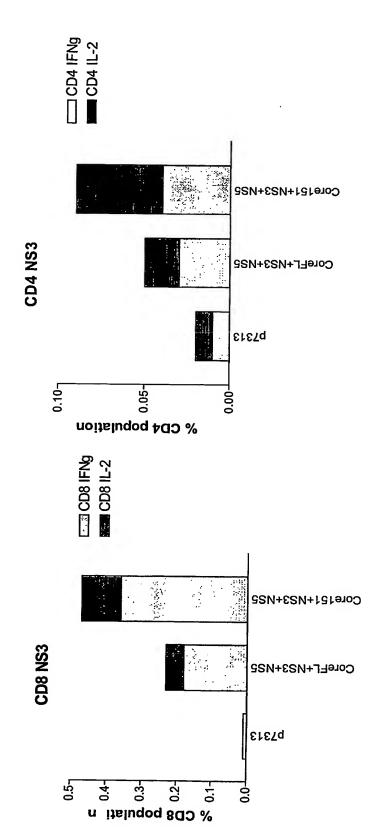


FIG. 22,

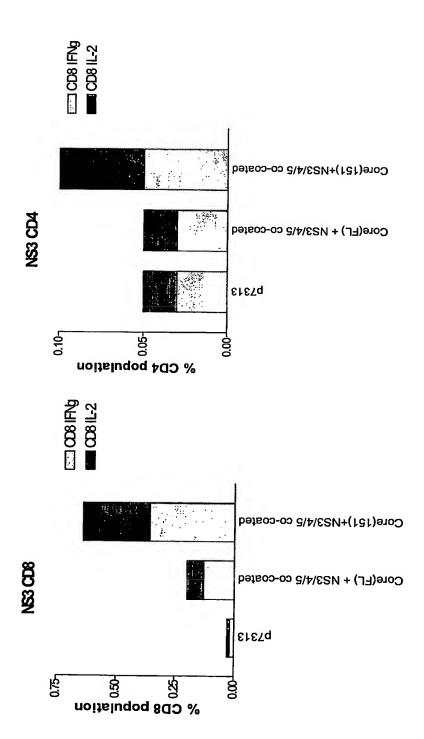


FIG. 23,